Summer 2011

Modeling Manduca sexta Gut Digestion and Absorption as a Plug Flow Reactor

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Abstract

A change in size can have a huge impact on an organism's digestive capabilities and consumption behavior. To explore this, we characterized the midgut of *Manduca sexta* as a plug flow chemical reactor and parameterized the gut with empirical data to see how different sizes affected reactor performance. Based on our model, we made predictions about how maximal absorption rate, optimal consumption rate, optimal gut content flow rate, gut passage time, and absorption efficiency scale with weight. The predicted optimal consumption rate scaled as weight to the 0.89 power, a good approximation of third, fourth, and fifth instar caterpillar food intake. The predicted maximal amino acid absorbance rate scaled as weight to the 0.74 power, which overestimated the observed amino acid assimilation to an increasing degree as the caterpillar grew. However, the observed dry growth scaled as weight to the 0.88, which was very close throughout ontogeny to the predicted maximal absorbance rate. This model offers insights into digestion in this insect; we believe our results support the hypothesis that *Manduca* optimize their behavior to maximize absorbance given their gut morphology.

Introduction

•As an organism increases in size, its surface area increases slower than its volume. This relationship between surface area and volume can impact an organism's behavior and physiology throughout its growth.

•Due to the complexity of biological systems mathematical models are needed to to gain simplified insight into the system. Models are useful based on their simplicity, accuracy, and the validity of their assumptions.

•The reactions pertaining to digestion and absorption within a simple, tubular gut can be characterized like a "plug flow reactor model" (Woods & Kingsolver 1999, see Model and Assumptions). Maximal absorbance rates can be determined for various gut sizes.

•We estimated the parameters of the model based on information about the tobacco hornworm, *Manduca sexta*, because they grow 10,000 fold in only 18 days. We also have extensive data with which we can compare our model's predictions and thereby test our model's accuracy.

Model

•As the caterpillar eats some "substrate" at some constant rate, it is digested by enzymes. We treat this breakdown as if it were in accordance with Michaelis-Menton saturation kinetics.

•As the "substrate" is broken down, "products" are formed which can then be absorbed along the boundary of the gut according to a different term obeying Michaelis-Menton saturation kinetics.

•These relationships can be formalized in the following system of ordinary differential equations (Woods and Kingsolver, 1999):

$$\frac{dS(x)}{dx} = \frac{1}{u}\left(\frac{V_{max}S(x)}{K_m + S(x)}\right)$$

$$\frac{dP(x)}{dx} = -\frac{1}{u}\left(\frac{2}{r}\frac{W_{max}P(x)}{L_m + P(x)} - \alpha\frac{V_{max}S(x)}{K_m + S(x)}\right)$$

•Within the model, absorbance rate is calculated in the following way:

$$AbsorbanceRate = 2\pi r\int_0^l \frac{W_{max}P(x)}{L_m + P(x)}dx$$

Table 1. Parameters and estimates based on *Manduca* Protein Breakdown

Model Parameter	Interpretation	Values/Units
S	(Initial) Substrate concentration*	$(31)\ mg \cdot ml^{-1}$
$\frac{dS}{dx}$	Change in substrate concentration with distance	$mg \cdot ml^{-1} \cdot cm^{-1}$
V_{max}	Max rate of substrate breakdown *	$500\ mg \cdot ml^{-2} \cdot h^{-1}$
K_m	Half-saturation constant (substrate) *	$1.5\ mg \cdot ml^{-1}$
P	(Initial) Product Concentration	$(0)\ mg \cdot ml^{-1}$
$\frac{dP}{dx}$	Change in product concentration with distance	$mg \cdot ml^{-1} \cdot cm^{-1}$
W_{max}	Max rate of product absorption †	$0.748\ mg \cdot cm^{-2} \cdot h^{-1}$
L_m	Half-saturation constant (product) ‡	$1.72\ mg \cdot ml^{-1}$
u	Gut content flow rate	$cm \cdot h^{-1}$
r	Gut radius §	cm
l	Gut length °	cm
α	Stochiometric conversion factor (substrate→ products)	1 —

* Woods & Kingsolver (1999) † Estimated from Woods & Chamberlin (1999) ‡ Estimated from Reuveni & Dunn (1994) § Unpublished data from Connell & Messerman (2011) ° Unpublished data from Raithel & Vela–Mendoza (2011)

Model Assumptions

•Our plug flow reactor describes continuous "plugs" moving through a constant sized tube with a constant velocity profile. A corollary of this is that there is no axial mixing, but there is perfect radial mixing.

•Breakdown from membrane bound peptidases and transport by transport proteins are treated as one step.

•Energetic costs of foraging, enzyme production, transport are ignored as are both diffusion and excretion.

•Gut parameters are constant through the length of the gut and through the development of the animal.

•Our model only examines digestion and absorbance in the context of proteins.

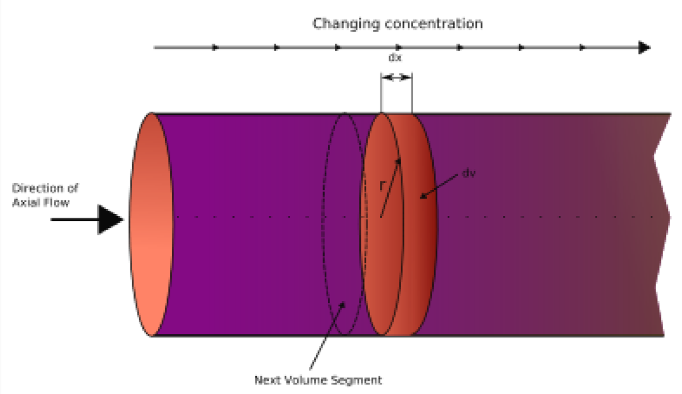


Fig. 1. A schematic of a plug flow reactor (http://en.wikipedia.org/wiki/Plug_flow_reactor_model).

Methods

•The system of differential equations were solved using a Dormand-Prince numerical approximation (Soetaert, et al. 2010) to give us the concentration profiles of "substrate" (S) and "product" (P) along the length of the gut.

•Absorbance rate was calculated using Simpson's Rule to evaluate the integral.

•By varying consumption rate and keeping all other parameters constant, we could see if there was a maximal absorbance rate, and if so, what the optimal flow rate was.

•Radius was found to be proportional to mass$^{0.36}$ by digitally calculating the cross sectional area of the gut and assuming the area was a circle (Fig. 2, unpublished data, Connell and Messerman, 2011).

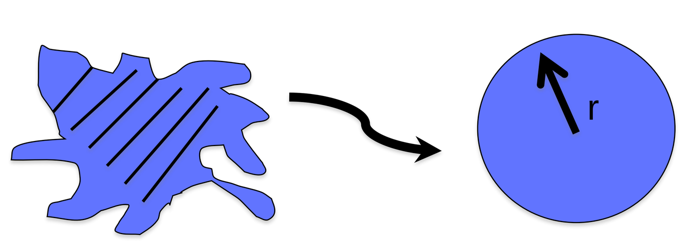
•Length was found to be proportional to mass$^{0.38}$ by using a caliper to measure midgut length of dissected *Manduca* of known weight (unpublished data, Raithel and Vela-Mendoza, 2011).

•With scaling relationships of radius and length to mass, we found the optimal flow rate and maximal absorbance rate for the different relevant sizes and aspect ratios of the gut.

•Along with maximal absorbance rate and optimal flow rate, we found optimal consumption rate by multiplying the optimal flow rate by the cross sectional area of the gut. Absorption efficiency is simply the maximal absorption rate divided by optimal consumption rate. Gut passage time is the gut length divided by the optimal flow rate at that length.

•Predictions were compared to relevant data gathered by Messerman and Sears (2009).

Fig. 2. Depiction of how the gut cross sectional area was used in our model. Note: our approach underestimates the actual lumen surface area.



Results

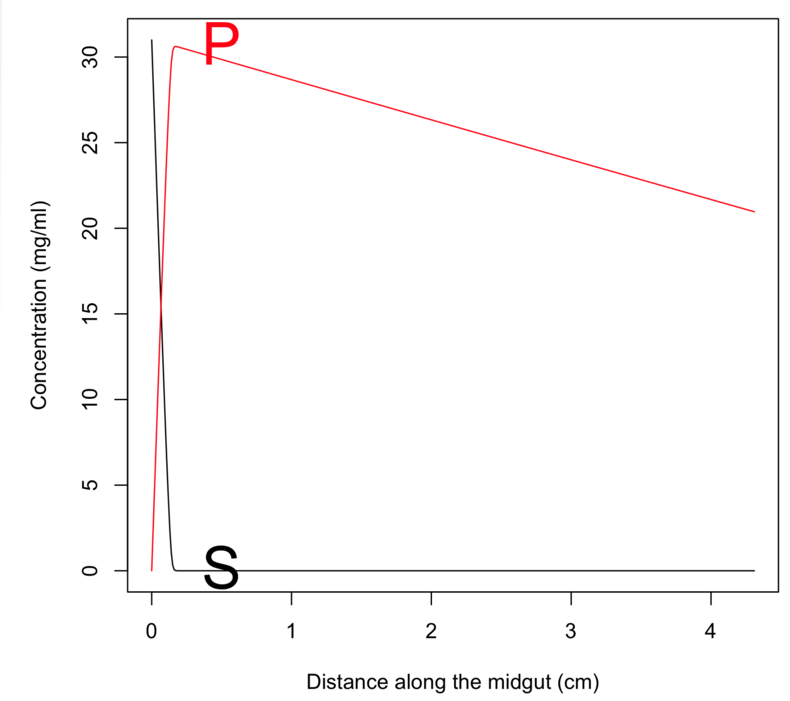


Fig. 3. The change in concentration of substrate (S) and product (P) along the distance of the midgut according to model predictions.

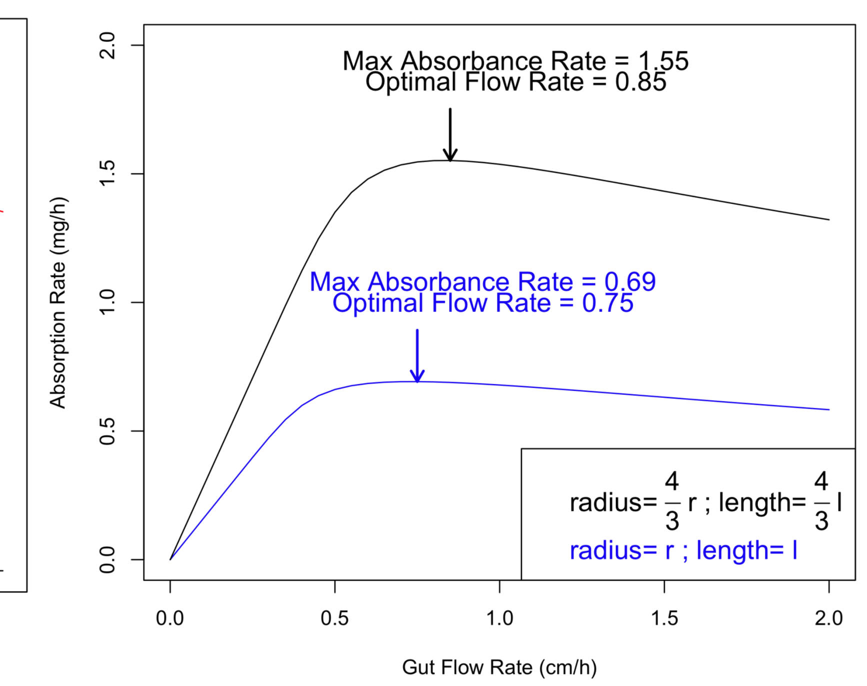


Fig. 4. An example of the absorbance rate as a function of gut flow rate for two different gut sizes. Parameters in this example were chosen to accentuate the maximal absorbance rate and optimal flow rate.

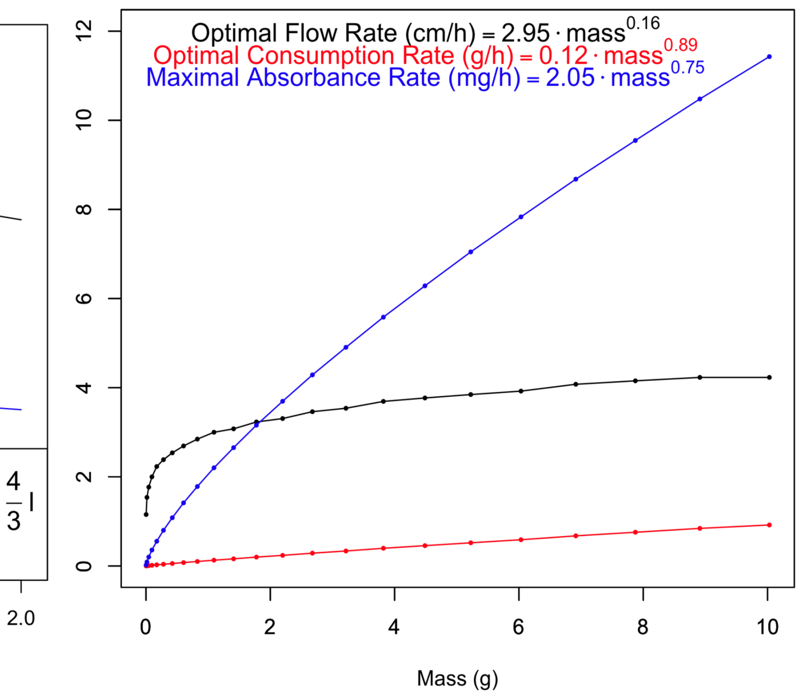


Fig. 5. The model's predictions of maximal absorbance rate, optimal consumption rate, and optimal flow rate as a function of mass.

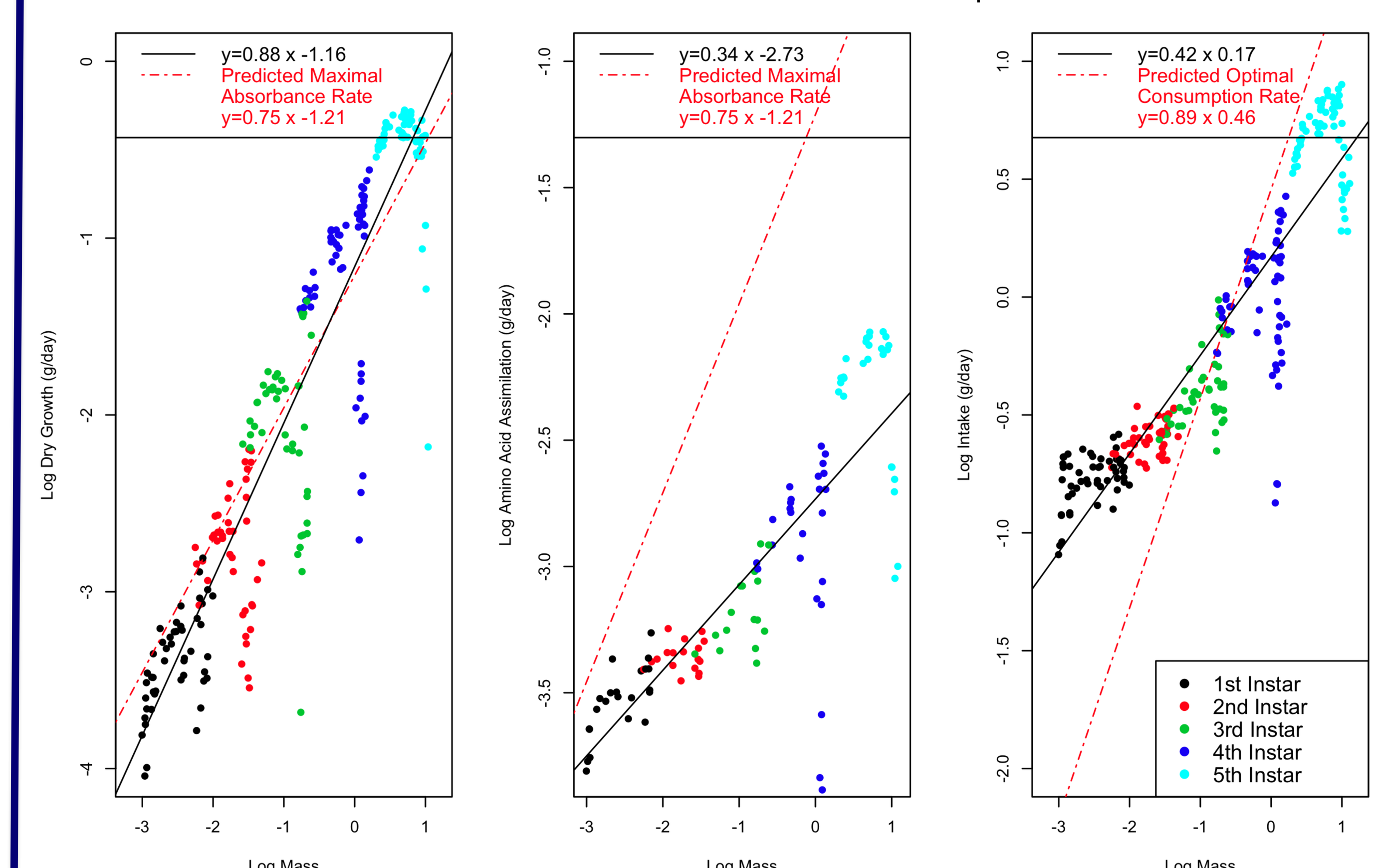


Fig. 6. Comparison of the model's prediction of optimal consumption rate to observed intake and predicted maximal absorbance to both dry growth and amino acid assimilation. All data is from unpublished data from Messerman and Sears (2009). Amino acid assimilation was estimated from the difference of nitrogen content between food and frass (Warner and James 2002).

•A maximal absorbance rate exists according to the model. This means that at some level of consumption, eating faster only serves to move food out of the digestive tract before the nutrients can be absorbed.

•Under an evolutionary optimization schema, the *Manduca* should be observed to eat close to the amount needed to optimize gut flow.

•As gut size increases, so too does maximal absorbance rate, optimal flow rate, and optimal consumption rate within the model.

•The *Manduca* eat more than the model predicts they should in the first two instars but less in the last two instars.

•Estimated amino acid absorption is much less than the predicted maximal absorbance rate after the first instar. Our model was a poor indicator of this parameter.

•Observed dry growth is very similar to the model's prediction of maximal absorption rate.

•The predicted optimal gut flow rate is several times higher than the estimated gut flow rate at most weights.

Table 2. The predicted scaling of measurable quantities based on the plug flow model.

Measurement	Predicted Scaling with Mass
Maximal Absorbance Rate $(g \cdot day^{-1})$	$0.05 \cdot \text{mass}^{0.75}$
Optimal Flow Rate $(cm \cdot h^{-1})$	$2.95 \cdot \text{mass}^{0.16}$
Optimal Consumption Rate $(g \cdot day^{-1})$	$2.87 \cdot \text{mass}^{0.89}$
Absorption Efficiency	$0.02 \cdot \text{mass}^{-0.14}$
Gut Passage Time $(hours)$	$0.70 \cdot \text{mass}^{0.22}$

Conclusions and Further Work

•For such a simplified model, the plug-flow reactor provides a fairly good approximation of gut digestion in *Manduca*. Perhaps some of our assumptions "cancel" each other out (e.g. neglect of diffusion and our underestimate of gut lumen surface area).

•The deviations from predictions we observe could be due to overly simple assumptions rather than inherent flaws of the plug-flow model. By refining the model to deal with more biological complexity (e.g., if parameters changed by section of the gut and weight or diffusion to microvilli or multiple nutrients were considered) we could further test if a plug-flow set up is appropriate.

•If we deem the model accurate, then our results strongly suggests that the caterpillars have optimized their consumptive behavior to take advantage of their gut morphology.

•As a generalized model, our work suggests that physiological and behavioral processes might compensate for scaling relationships in a complicated fashion (e.g. maximal absorbance rate did not scale as mass$^{2/3}$).

Acknowledgments:
I would like to thank the entire Manduca Instar Team, both past and present with a special thanks to Dr. Kerkhoff for his mentorship. This project was funded by Kenyon Summer Science Scholars and an NSF grant (DMS-0827208).

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